HM5 Precision and Accuracy:

Initial Validation and Verification of VETSCAN® HM5 Hematology Analyzer for Analysis of Canine, Feline, and Equine Blood Samples

Michelle Larsen, DVM

INTRODUCTION

The VETSCAN HM5 is a fully automated, point-of-care hematology analyzer that generates a 22-parameter, five-part differential complete blood count (CBC) with cellular histograms. The HM5 uses a combination of impedance, chemical differentiation, and species-specific software technologies using a small sample volume (50 µL) of anticoagulated blood (EDTA) to generate results in under 4 minutes. Impedance technology is used to count red blood cells (RBC), white blood cells (WBC) including differential counts, and platelets (PLT) based upon cell size. Spectrophotometry is used to directly measure hemoglobin (HGB). These hematology technologies are common and have been incorporated into a wide range of instrument designs.[1] Upon market introduction of the VETSCAN HM5, a multi-center study was performed to assess the precision and accuracy of the analyzer for canine, feline and equine species. Subsequently, for the launch of the updated VETSCAN HM5 in 2013, a smaller internal study was performed to demonstrate equivalency between the two models. Both studies will be discussed to demonstrate the competency of the VETSCAN HM5.

INITIAL HM5 VALIDATION

MATERIALS & METHODS

Samples

Anticoagulated (EDTA) blood samples from three different institutions were obtained from both clinically ill and healthy dogs (n=279), cats (n=189), and horses (n=151), representing various breeds, ages, and sexes.[2] Samples included in the study were collected at the William R. Pritchard Veterinary Teaching Hospital, University of California Davis (UCD); the James L. Voss Veterinary Teaching Hospital, Colorado State University (CSU); and the Veterinary Teaching Hospital at Michigan State University (MSU). Patient samples were consecutively presented between August 2007 and September 2007. Blood was primarily obtained from canine and feline jugular, cephalic, or medial saphenous veins and equine jugular veins. Samples were stored for <4 hours at room temperature or <8 hours at 4°C and brought to room temperature then gently mixed at least 10-15 times prior to analysis.[2]

VETSCAN HM5
Launched in 2008

VETSCAN HM5
Launched in 2013

Instruments[2]

The HM5 analyzer was evaluated for precision and accuracy against the ADVIA® 120 (Bayer Corporation, Tarrytown, NY, USA), a flow cytometry-based analyzer, which was designated as the reference standard for comparison. The ADVIA 120 has been validated for diagnostics in many veterinary species, and is used in university, commercial, and research veterinary clinical pathology laboratories.[3,4] Directly measured data (RBC, HCT, PLT, HGB, and WBC with differential) were compared for both analyzers. Manual differential cell counts were performed to confirm automated differentials and to further investigate samples with poor leukocyte correlation between the two analyzers.

Statistical Analysis

Coefficients of Variation (CV) and standard deviations (SD) were generated for the precision study. Accuracy data generated by both hematology analyzers as well as leukocyte differentials between manual and automated methods were compared by Spearman rank correlation coefficient (r_s) and then squared, Passing-Bablok regression analysis and Bland-Altman plots, using a commercially available computer statistical software program (Analyze-it, Analyze-it Software Ltd., Leeds, UK).[2] Correlation was considered "excellent" for (R^2) = 0.86-0.99, "good" for (R^2) = 0.64-0.85, "fair" for (R^2) = 0.35 – 0.62 and "poor" for (R^2) <0.34.[5]

RESULTS

Precision[6]

Ten replicates of three control levels (low, medium, high) were run over five consecutive days at all three test sites and data merged for analysis. The HM5 demonstrated good reproducibility at all control levels on all three analyzers located at the different sites. There was no CV% greater than 10% and the CVs for all analytes at high, normal, and low control levels were less than 5%, except for platelets using the low control and monocytes at all control levels (see Table 1).[6, 7] The higher PLT CV% at the low control level can be attributed to the intrinsic variation of counting low numbers of small volume events by impedance analysis and is a known disadvantage of using CV%.[8] In addition, when patient PLT levels are below the reference range for any analyzer, a blood smear should always be performed for verification.[1] Monocyte (MON) precision did not statistically affect lymphocyte (LYM) or neutrophil (NEU) precision and is unlikely to significantly alter medical decisions.

Table 1.
Multi-Site HM5 Precision Data [6]

	Control Levels CV% (±SD)		
Analyte	**High Level**	**Normal Level**	**Low Level**
WBC	1.37 (0.26)	1.70 (0.14)	2.65 (0.09)
NEU	2.36 (0.17)	2.39 (0.10)	3.16 (0.07)
LYM	4.10 (0.46)	3.73 (0.12)	4.71 (0.04)
EOS	2.02 (0.14)	2.14 (0.09)	3.13 (0.07)
HGB	1.21 (2.04)	1.48 (1.87)	2.15 (1.29)
RBC	1.69 (0.09)	1.73 (0.08)	2.08 (0.05)
PLT	2.64 (13.43)	3.74 (8.75)	8.89 (6.41)

Units for standard deviation (SD) are: WBC, NEU, EOS, LYM, PLT: 10^9/L; RBC 10^12/L; HGB mmol/L

Clinical Correlation[9, 10, 11]

Excellent correlation was found for WBC, LYM, NEU, RBC and HGB for all species, EOS for horses and cats, and PLT for dogs. Fair correlation was found for PLT for equine and feline, and EOS for dogs. Fair PLT correlations for both cats and horses is likely due to species-specific clumping properties of PLT, (see feline discussion below). Most clinicans are aware of these species' PLT properties and a manual smear should be performed to confirm a low automated PLT count. MON statistical correlation was uniformly poor for all species between the analyzers and remained poor when correlation for both analyzers was compared to manual differentials. Correlations between the HM5 and Advia for EOS assessment ranged from excellent for horses and cats to fair for dogs, likely due to five poorly correlated, high EOS counts for dogs, as a few clinically significantly increased canine EOS values (>1,000/µL) were seen within the populations. Larger numbers of canine samples with increased EOS percentages are needed to better assess correlation and agreement.

Discussion

Most canine and equine analytes had fair to excellent correlation with no to minor proportional and constant biases, as measured by Passing-Bablok slope and Bland Altman plots, respectively.[9, 10] (See Figures 1a-1d) Taken as a whole, these small biases are very unlikely to impact the medical decisions when comparing the results between the VETSCAN HM5 and ADVIA 120 hematology analyzers.

Table 2.
Spearman's Rank Correlations: HM5 vs. Advia 120

Canine[10]

Analyte	R^2	p-value
WBC	0.99	<0.0001
LYM	0.97	<0.0001
NEU	0.86	<0.0001
RBC	0.95	<0.0001
HGB	0.97	<0.0001

Feline[11]

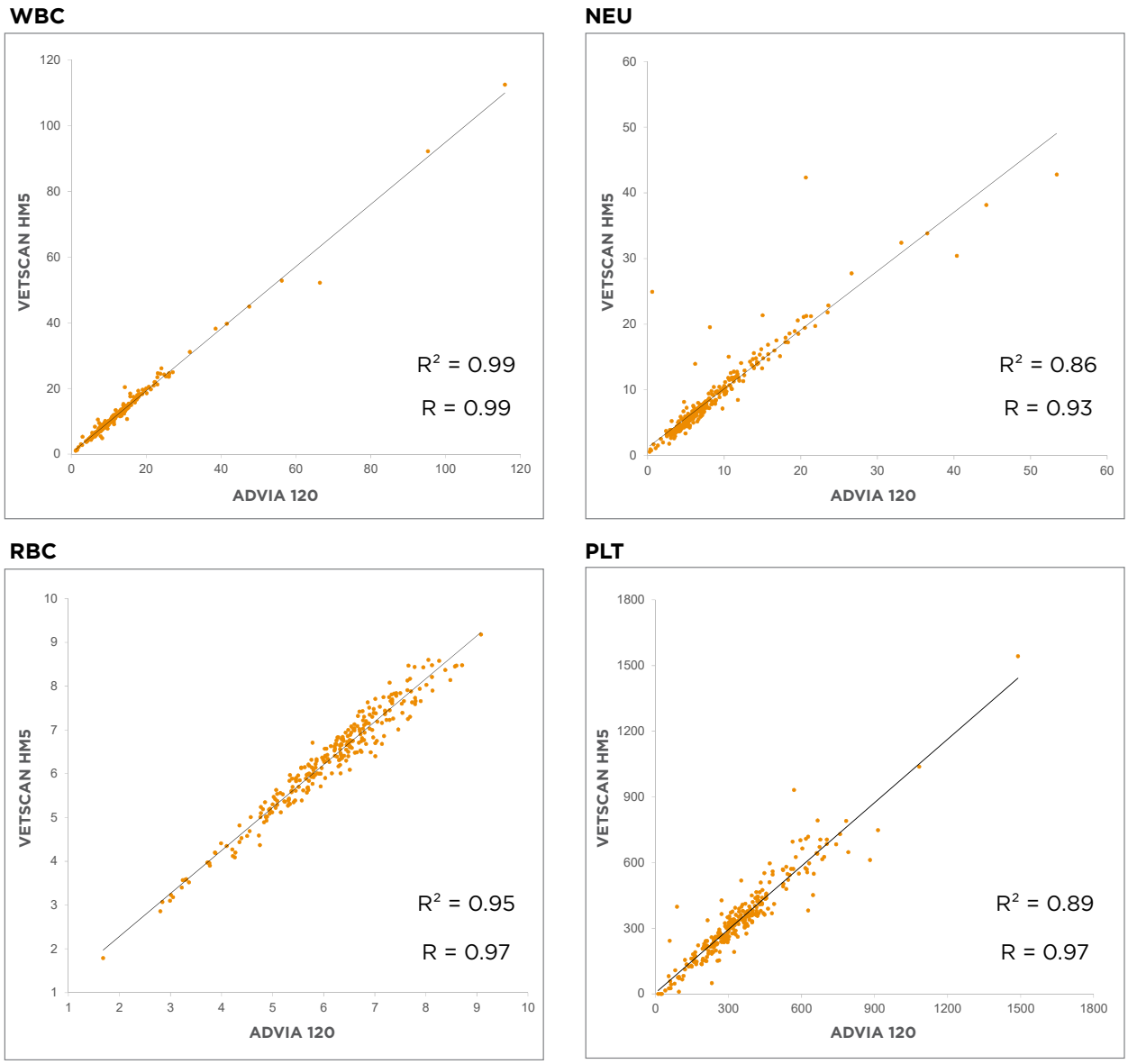
Analyte	R^2	p-value
WBC	0.89	<0.0001
LYM	0.87	<0.0001
NEU	0.86	<0.0001
RBC	0.97	<0.0001
HGB	0.98	<0.0001

Equine[9]

Analyte	R^2	p-value
WBC	0.98	<0.0001
LYM	0.91	<0.0001
NEU	0.97	<0.0001
RBC	0.93	<0.0001
HGB	0.97	<0.0001

All feline analytes had fair to excellent correlation, except for MON and PLT, but there was evidence of medically relevant proportional and constant biases for a few analytes.[11] The majority of these biases are most likely associated with either large PLT or small to large PLT aggregates being counted as other cell types (RBCs and WBCs), on the HM5. It is well-known in the veterinary community that feline PLT can occasionally have similar cellular volume as feline RBCs, and that feline platelets are prone to aggregation during blood collection.[12] In the study population, feline macroplatelets and platelet aggregation are likely responsible for the negative proportional and constant biases observed with HM5 PLT counts, and likely contribute to the positive proportional and constant biases observed for WBC, LYM, MON, NEU counts. Careful analysis of feline WBC and PLT counts along with histograms will most often identify these occurrences and indicate the need for blood smear review, blood redraw, or medical decision adjustment.

Figure 1. Clinical Correlation - Canine



Figures 1a-d: Spearman Rank Correlation Plots between the VETSCAN HM5 and Advia 120 for 279 canine samples, aggregated among the three universities. Units are: WBC, NEU, PLT: 10^9/L; RBC 10^12/L

INITIAL VETSCAN HM5 VALIDATION STUDY SUMMARY

The VETSCAN HM5 demonstrated acceptable performance compared to the ADVIA 120 commercial hematology analyzer, validated for diagnostics, with samples taken from the general patient population at three different university veterinary hospitals.

Instrument precision at low, normal, and high control values for almost all analytes was excellent. Most canine, feline and equine analytes had excellent correlations, as defined by R^2, to the ADVIA 120 reference lab analyzer.

For canine and equine species, there were minor to no proportional and constant biases. For feline species, few analytes did have proportional and constant biases, the majority of which can be associated with platelet clumping/aggregates inherent in feline blood.

HM5c EQUIVALENCY STUDY

In 2012, the VETSCAN HM5 was remodeled for improved user interface with color touchscreen display and maintenance requirements; however, the technologies of impedance, spectrophotometry, and species-specific software remained. An internal verification study was conducted to demonstrate precision performance for the new HM5 model (termed HM5c for comparative purposes only) and performance equivalency across both HM5 models using R^2 correlation for canine, feline, and equine species.

RESULTS

Precision

Precision was evaluated on one VETSCAN HM5c on the same day using low (n=20), normal (n=22), and high (n=22) control levels. Results are shown in Table 3.

Table 3.
HM5c Precision Data[7]

	Control Levels CV% (±SD)		
Analyte	**High Level**	**Normal Level**	**Low Level**
WBC	0.8 (0.15)	1.24 (0.10)	2.41 (0.09)
NEU	2.37 (0.16)	1.67 (0.07)	4.50 (0.09)
LYM	4.93 (0.57)	3.39 (0.11)	3.56 (0.05)
EOS	1.45 (0.11)	1.67 (0.08)	2.95 (0.07)
HGB	1.37 (2.25)	0.93 (1.16)	1.16 (0.67)
RBC	1.25 (0.06)	3.29 (0.15)	1.44 (0.04)
PLT	2.94 (16.67)	4.17 (9.90)	5.78 (4.26)

Units for standard deviation (SD) are: WBC, NEU, EOS, LYM, PLT: 10^9/L; RBC 10^12/L; HGB mmol/L

Clinical Correlation[5, 13]

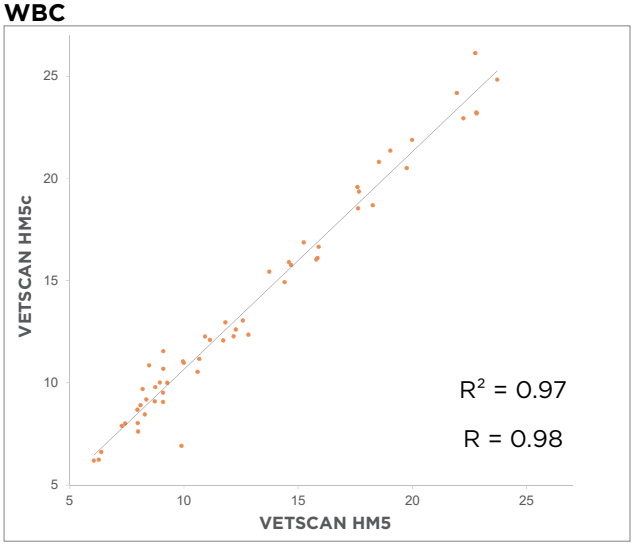
On a single day, at a single facility, canine (n=55) and feline (n=86) clinical samples were run in duplicate and simultaneously on one VETSCAN HM5 and one VETSCAN HM5c analyzer.[13] Correlation was considered "excellent" for (R^2) = 0.86-0.99, "good" for (R^2) = 0.64-0.85, "fair" for (R^2) = 0.35 – 0.62 and "poor" for (R^2) <0.34.[5] Selected correlation graphs shown in Figures 2 and 3.

For canine samples, correlations for most major parameters (WBC, NEU, HGB, EOS, and PLT) were excellent, ranging from 0.94-0.98. Canine RBC and MCV correlations were good, 0.92 and 0.90, respectively.[13] LYM had a fair correlation.

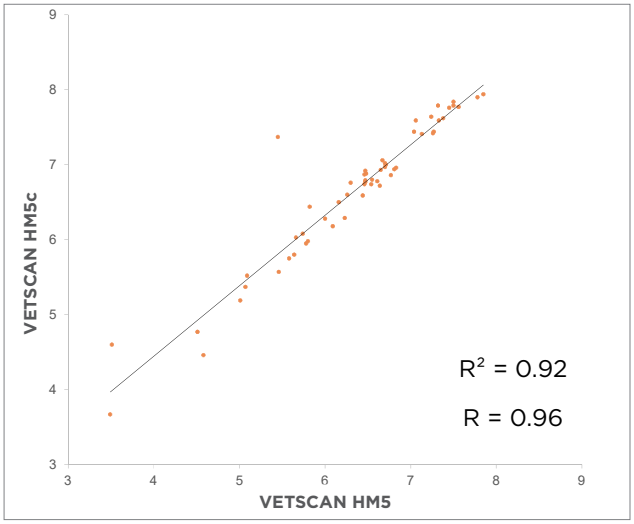
For feline samples, correlations for all major parameters (WBC, RBC, NEU, MCV, HGB, EOS and PLT) were excellent, ranging from 0.93-0.99.[13] Similar to canine correlations, LYM had a fair correlation.

Figure 2. HM5c Clinical Correlation - Canine

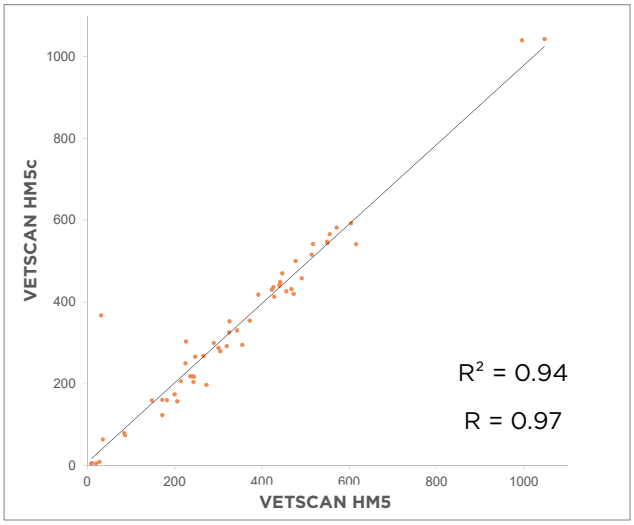
WBC



RBC



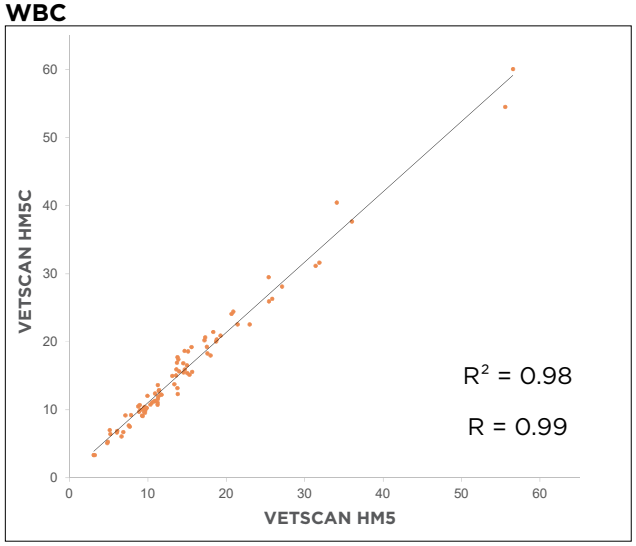
PLT



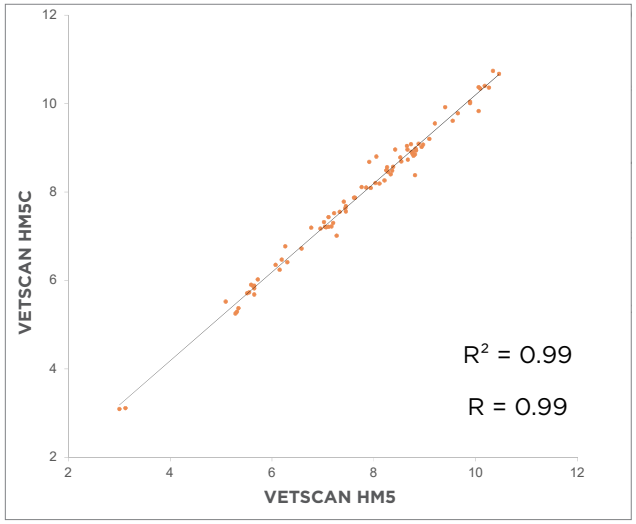
Figures 2a-c: Equivalency Correlation Plots between the VETSCAN HM5 and VETSCAN HM5c, for 55 canine samples. Units are: WBC, NEU, PLT: 10^9/L; RBC 10^12/L

Figure 3. HM5c Clinical Correlation - Feline

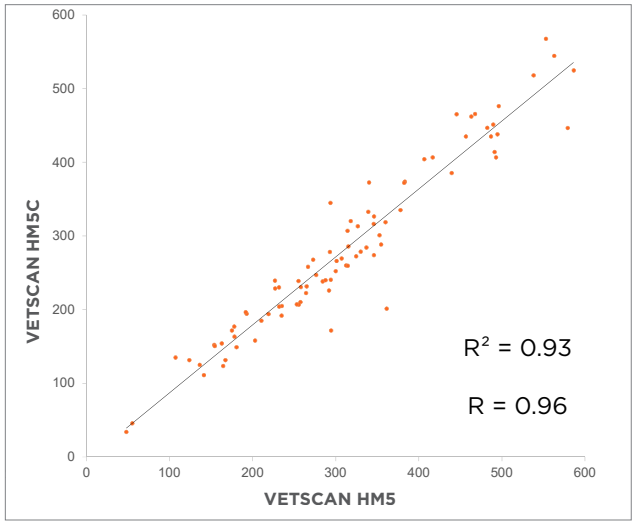
WBC



RBC



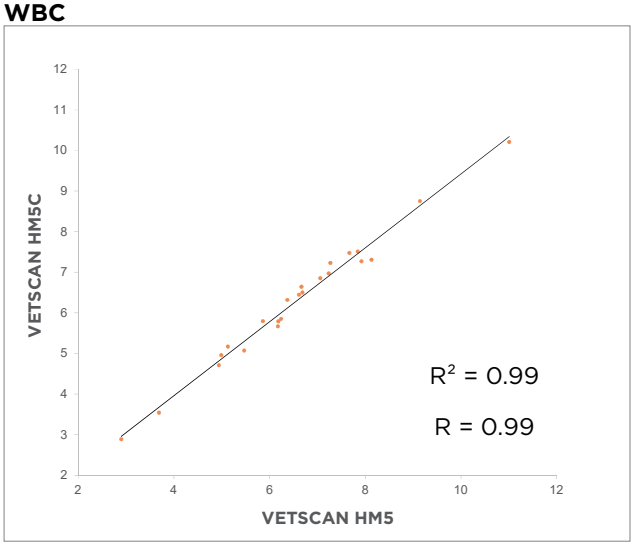
PLT



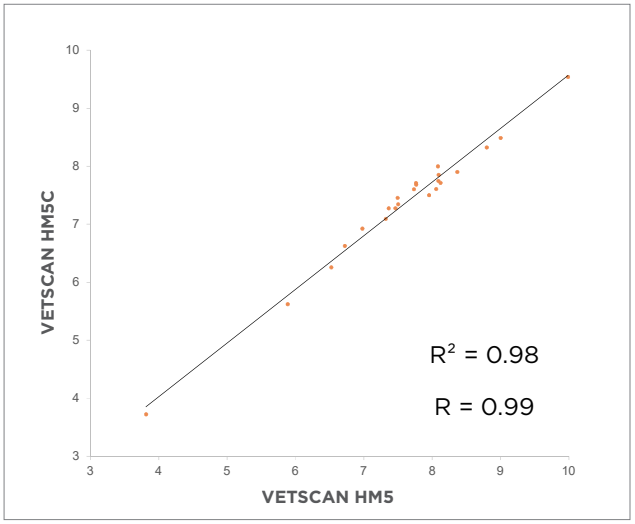
Figures 3a-c: Equivalency Correlation Plots between the VETSCAN HM5 and VETSCAN HM5c, for 86 feline samples. Units are: WBC, NEU, PLT: 10^9/L; RBC 10^12/L

Figure 4. HM5c Clinical Correlation - Equine

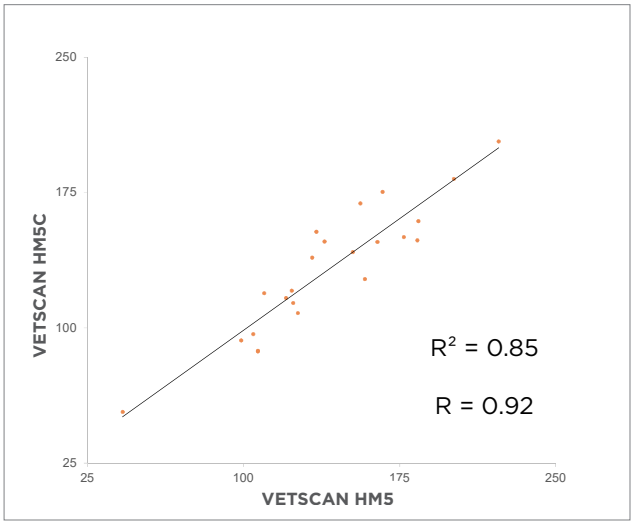
WBC



RBC



PLT



Figures 4a-c: Equivalency Correlation Plots between the VETSCAN HM5 and VETSCAN HM5c, for 23 equine samples. Units are: WBC, NEU, PLT: 10^9/L; RBC 10^12/L

Equine correlation and precision were performed on a single day, at a single lab facility, with 5 replicate runs simultaneously on one VETSCAN HM5 and two VETSCAN HM5c analyzers.[14] All HM5c CV% were <10%, except for MON (possibly due to medically relevant proportional and constant biases, as discussed above in the Initial HM5 Verification section). Correlation defined by R^2 for both HM5 analyzer models were excellent for all major parameters, except for PLT (R^2 =0.85, good).[14] See Figures 4a-c.

EQUIVALENCE STUDY SUMMARY

Both precision and correlation for the VETSCAN HM5c, when compared with the validated VETSCAN HM5, performed in equivalence for canine, feline and equine species with excellent precision and excellent correlation for most analytes for canine, feline, and equine species.

CONCLUSION

The VETSCAN HM5 series of hematology analyzers were precise and perform well when compared to the ADVIA 120 reference hematology analyzer for the measurement of canine, feline, and equine hematologic analytes.

Initial HM5 validation study performed by:

1. Keith DeJong, DVM and Sean Owens, DVM, DACVP, Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, California
2. Carrie Flint, DVM and Cheryl Swenson, DVM, DACVP, Department of Pathobiology and Diagnostic Investigation and Diagnostic Center for Population and Animal Health, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan
3. Linda Vap, DVM, DACVP, Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Ft. Collins, Colorado

HM5c Equivalence Study performed by: Lawrence Lem, PhD (Abaxis)

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1. Thrall. General Principles of Laboratory Testing and Diagnosis. Veterinary Hematology and Clinical Chemistry. 2nd edition p. 14-16. **2.** Protocol on File. Study report number TI-05506. Zoetis Inc. **3.** Giordano A, Rossi G, Pieralisi C, Paltrinieri S. Evaluation of equine hemograms using the ADVIA 120 as compared with an impedance counter and manual differential count. Vet Clin Pathol. 2008;37:21-30. **4.** Moritz A, Fickenscher Y, Meyer K, Failing K, Weiss DJ. Canine and feline hematology reference values for the ADVIA 120 hematology system. Vet Clin Pathol. 2004;33:32-38. **5.** Bauer, N. et al. Evaluation of the automated hematology analyzer Sysmex XT-2000iV compared to the Advia 2120 for its use in dogs, cats, horses: part I- precision, linearity, and accuracy of complete blood cell count. J Vet Diag Investig 23 (6), 1168-1180, 2011. **6.** Data on File. Study report number TI-05688. Zoetis Inc. **7.** Data on File TI-05505 **8.** Reed GF, Lynn F, Meade BD. Use of coefficient of variation in assessing variability of quantitative assays. Clin Diagn Lab Immunol. 2002;9:1235-1239. **9.** Data on File. Study report number TI-05509. Zoetis Inc. **10.** Data on File. Study report number TI-05508. Zoetis Inc. **11.** Data on File. Study report number TI-05510. Zoetis Inc. **12.** Harvey, John W. Veterinary Hematology. Elsevier, 2012, p. 17-18. **13.** Data on File. Study report number TI-05504. Zoetis Inc. **14.** Data on File. Study report number TI-05507. Zoetis Inc.